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Research Article

Synthesis, characterization, and electroanalytical investigation of copper-based materials with antibacterial investigation

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ABSTRACT

Copper-amino acid-based materials have attracted significant interest because of their unique physicochemical, electrochemical, and biological properties. In this work, a copper(II)-cysteine coordination product and L-cysteine-capped copper nanoparticles (L-Cys-CuNPs) were prepared using a simple wet-chemical method. Cyclic voltammetry revealed that L-Cys-CuNPs exhibited more pronounced redox behaviour and better electron-transfer kinetics than the copper-cysteine coordination product. UV-visible spectroscopy confirmed copper-cysteine coordination and nanoparticle formation through characteristic absorption (580 nm) features. FTIR analysis showed that copper and cysteine interacted strongly via thiols (-SH) and carboxylate (COO⁻) groups, thus confirming that the copper nanoparticles were effectively surface-capped with L-cysteine. X-ray Diffraction (XRD) results showed that the copper-cysteine coordination product was mostly amorphous. In contrast, the L-Cys-CuNPs showed sharp diffraction peaks at $2\theta = 43.1, 50.1, \text{ and } 73.8$, indicating crystalline copper. Scanning electron microscopy (SEM) images showed that the copper-cysteine coordination product had an irregular shape. The L-Cys-CuNPs appeared well dispersed, nearly spherical, and showed less clumping. EDS analysis showed C, N, O, and Cu in the Cu(II)-cysteine coordination product and L-cysteine-capped copper nanoparticles. The elemental composition supports the incorporation of cysteine-associated elements with copper in the synthesized material. Antibacterial tests on selected Gram-positive *Staphylococcus aureus* (*S. aureus*) and Gram-negative *Escherichia coli* (*E. coli*) showed that L-Cys-CuNPs had stronger antibacterial activity.

INTRODUCTION

The interaction of transition metal ions with biologically relevant ligands is an important area of electroanalytical, coordination, and bioinorganic chemistry. [1] These interactions are central to bioinorganic, coordination, and electroanalytical studies. They are also strongly involved in biological processes such as enzymatic catalysis, metal ion transport, and electron-transfer mechanisms. [1] Copper is an important transition metal to study because it can exist in two oxidation states (Cu⁺¹ and Cu⁺²) and has a strong ability to form bonds with other molecules. As a result, copper is widely studied in metal-ligand interaction systems and their influence on electrochemical properties. [2]

L-cysteine, a sulfur-containing amino acid, acts as an effective ligand for copper because of its thiol (-SH), amino (-NH₂), and carboxyl (-COOH) groups. [3] Among these, the sulfur atom plays an important role in coordination, especially with soft and borderline metal ions such as copper. [3] Because of its sulfur and nitrogen donor atoms, L-cysteine forms stable chelate complexes, which govern the electronic structure and redox behaviour of copper. [2] These copper-cysteine interactions are biologically relevant; they replicate the copper environment found in proteins and enzymes. [1] Electroanalytical methods, especially cyclic voltammetry (CV), are effective tools for investigating redox behaviour and complex formation

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in metal-ligand systems. ^[4] Cyclic voltammetry provides insights into electron-transfer mechanisms, redox reversibility, and how ligand coordination affects peak potentials (E_{pa}, E_{pc}) and peak currents (I_{pa}, I_{pc}). ^[4] Copper-cysteine binding is usually indicated by changes in peak potentials, peak currents, and peak separation (ΔE_p), reflecting changes in electron-transfer kinetics and thermodynamic stability. ^[5] Medium pH significantly affects the electrochemical behaviour of the copper-cysteine coordination product. ^[6] A primary factor in cysteine metal binding is its protonation-deprotonation equilibrium. Under acidic conditions, protonation of the thiol and amino groups reduces metal-binding ability, yielding weaker complexes. ^[3] In contrast, deprotonation of the thiol group at neutral or alkaline pH forms thiolate (S⁻), which has a strong affinity for copper ions and significantly enhances complex stability. These pH-dependent changes in ionic species relate to alterations in voltammograms and may be used to identify species types, coordination modes, and stability. Electrochemical studies employ a range of physicochemical methods to confirm complex formation and to characterize structural, optical, and morphological properties. These methods include UV-visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and scanning electron microscopy (SEM). ^[7] UV-visible spectroscopy primarily studies electron transitions, whereas FTIR analysis confirms vibrational changes associated with coordination, including the functional groups involved in bonding. ^[7] Copper-cysteine coordination products have received significant attention due to their notable biological activity, particularly their antibacterial properties. ^[8] Copper-based materials have been reported to exhibit antibacterial activity through several possible mechanisms, including interactions with bacterial cell surfaces and oxidative-stress-related processes. However, the relative contribution of these mechanisms depends on the physicochemical properties of the copper-containing materials and experimental conditions. The stability and availability of copper in the copper-cysteine coordination product may influence its interaction with bacterial cells and, consequently, its antibacterial activity. ^[8] The present study focused on a detailed electroanalytical study of the copper-cysteine coordination product using cyclic voltammetry, supported by comprehensive physicochemical characterization and evaluation of its biological activity.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals in this study were analytical reagent (AR) grade and used as received, without further purification. All solutions were prepared with double-distilled or deionized water. Copper chloride dihydrate (CuCl₂·2H₂O)

acted as the source of the metal, and L-cysteine (C₃H₇NO₂S) was employed as both the ligand for complexation and as the capping agent. Hydrazine hydrate (N₂H₄·H₂O) served as the reducing agent for making copper nanoparticles.

Synthesis of copper-cysteine coordination product

A 0.04 M sodium acetate buffer was prepared using deionized water, sodium acetate, and acetic acid. The pH of the reaction medium was adjusted to 6.0 by adding dilute sodium hydroxide to increase the pH or hydrochloric acid to decrease it. This provided a controlled aqueous medium for synthesis of the copper-cysteine coordination product. ^[9] To prepare the copper-L-cysteine coordination product, 10 mL of 0.001 M copper chloride dihydrate (CuCl₂·2H₂O) solution (Fig. 1a) was gradually added to 10 mL of 0.002 M L-cysteine (C₃H₇NO₂S) (Fig. 1b) under constant stirring. ^[10] Copper precursor and L-cysteine were used in amounts of 1.0×10^{-5} mol and 2.0×10^{-5} mol, respectively, resulting in an initial Cu(II) to L-cysteine molar ratio of 1:2. The reaction mixture had a total volume of 20 mL before further treatment. Upon mixing, a greyish-violet precipitate (Fig. 1c) formed within 5 min, indicating coordination between the copper ion and L-cysteine. The colloidal aqueous solution of the copper-cysteine coordination product was prepared by placing the reaction mixture on a magnetic stirrer at 60°C and 400 rpm for 4 hours to achieve complete complexation and uniform particle formation. ^[11] After the reaction, the precipitate was collected and centrifuged at 7500 rpm for 30 minutes. ^[11] The solid was washed twice with ethanol, then again with deionized water to remove unreacted substances and residual impurities. The purified precipitate was then dried in a furnace at 60°C for 12 hours to produce a fine powder. This dried material was used for further characterization and is called the isolated copper-cysteine coordination product.

Synthesis of L-cysteine-capped copper nanoparticles

The synthesis of L-Cys-CuNPs was carried out following a procedure similar to that used in the formation of the copper-cysteine coordination product in acetate buffer at pH 6.0. The pH was adjusted to 6.0 using sodium hydroxide to increase it and hydrochloric acid to decrease it. Subsequently, 10 mL of 0.001 M copper chloride

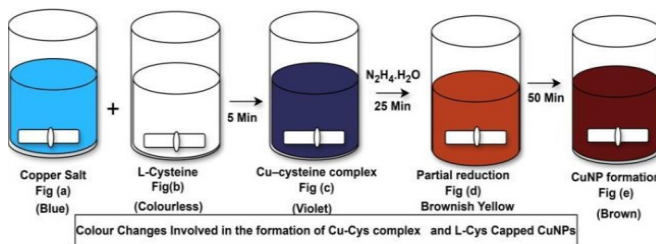


Fig. 1: Formation of Cu(II)-cysteine coordination product and L-cysteine-capped copper nanoparticles



dihydrate ($\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$) solution (Fig. 1a) was gradually added to 10 mL of 0.002 M L-cysteine ($\text{C}_3\text{H}_7\text{NO}_2\text{S}$) (Fig. 1b) under constant and continuous stirring.^[10] The initial Cu(II)-L-cysteine mole ratio was 1:2, and the reaction volume was 20 mL before the addition of the reducing agent. Hydrazine hydrate ($\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$, 80%) was used as the reducing agent. A diluted hydrazine working solution was prepared using deionized water. Subsequently, 2 mL of the freshly prepared hydrazine working solution was added to the 20 mL aqueous copper-cysteine reaction mixture under continuous stirring. After adding the diluted hydrazine solution, the total reaction volume was approximately 22 mL. The reaction mixture was maintained at 60°C under continuous stirring at 400 rpm in an open atmosphere. The formation of L-cysteine-capped copper nanoparticles was indicated by the gradual color change of the reaction mixture from brownish-yellow to reddish-brown. A brownish-yellow solution was formed after 25 minutes.^[12] After 50 minutes, the mixture turned reddish-brown (Fig. 1e), confirming the reduction of ionic copper to metallic copper nanoparticles.^[12,13] The reaction mixture was collected, washed twice with ethanol, and rinsed twice with water to remove any unreacted species.^[12] The resulting precipitate was dried in a furnace at 60°C for 12 hours to remove moisture and produce powdered metallic nanoparticles. The formation and properties of the copper nanoparticles were then evaluated using suitable characterization techniques. The observed color change was considered preliminary evidence of nanoparticle formation, while instrumental characterization results assessed the metallic nature and structural characteristics.

Instrumentation and Software

Characterization Techniques

Electrochemical interactions were studied by potential sweep (forward and backward) of copper-cysteine. Cyclic voltammetry (CV) was performed using a STAT-20 (EC-Proyog, India) with a glassy carbon working electrode, a platinum counter electrode, and an Ag/AgCl reference electrode. Measurements were conducted over -0.6 to 1.0 V at a scan rate of 60 mV s^{-1} .

Antibacterial activity

The antibacterial properties of the Cu(II)-cysteine-coordinated complex and L-cysteine-capped copper nanoparticles were examined against the Gram-negative bacterium *Escherichia coli* and the Gram-positive bacterium *Staphylococcus aureus* using the agar well diffusion method. Sterile Mueller-Hinton agar plates were inoculated with freshly prepared bacterial cultures under aseptic conditions. Wells about 6 mm in diameter were made with a sterile cork borer, and equal volumes of the samples were added. The plates were incubated at 37°C for 24 hours to allow bacterial growth and sample diffusion

through the agar. After incubation, the diameter of the clear inhibition zone around each well was measured in millimetres using a transparent ruler or vernier calliper. The inhibition zone diameter assessed the antibacterial activity of the materials. ZOI measurements were taken from three independent experiments ($n=3$), and results are given as the mean $\pm$ standard deviation (SD).

RESULTS AND DISCUSSION

Electrochemical Characterization

Cyclic Voltammetry Studies (CV)

In this work, the interaction between L-cysteine and copper ions was studied using cyclic voltammetry (CV) in a slightly acidic medium (pH 6.0) in sodium acetate buffer. Copper in the sodium acetate buffer shows a significant anodic peak potential (E_{pa} , 0.318 V) and a cathodic peak potential (E_{pc} , -0.120 V).^[4-6] Upon the gradual addition of L-cysteine (0.0 to 0.5 mL), a progressive potential (E_{pa} , 0.318 V) shift in the anodic peak potential (E_{pa}) from 0.318 V to 0.191 V was observed (Fig. 2). This shift indicates strong coordination with the copper ion and the thiol (-SH) group of L-cysteine, leading to the formation of a copper-cysteine coordination product.^[2,13,14] A decrease in peak potential indicates that complex formation altered the copper-ion electrochemical environment. Furthermore, adding L-cysteine reduced the availability of free copper ions in aqueous solution, altering the system's redox behaviour and electron transfer.^[15] A gradual decrease in the peak potential was observed with increasing L-cysteine concentration (0.0-0.5 mL). Table 1 shows the observed peak potential and peak current, indicating reduced concentration and diffusion of electroactive copper species. In addition, the anodic oxidation peak becomes broader and less intense with increasing ligand concentration, suggesting partial passivation due to adsorption of copper-cysteine species on the electrode.^[4]

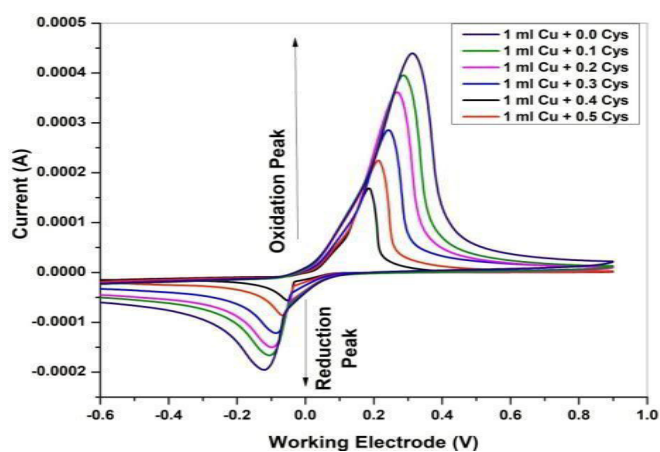


Fig. 2: Interaction of copper with cysteine in cyclic voltammetry with a scan rate of 60 mV s^{-1} .

Table 1: Interaction of copper with cysteine in cyclic voltammetry, with peak potential and peak current in sodium acetate buffer solution, pH 6.

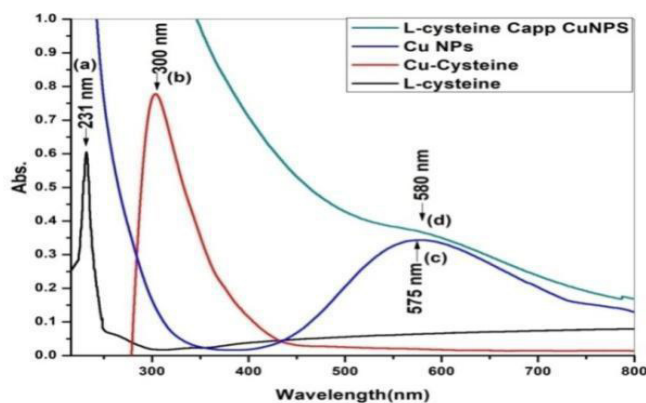
Copper–cysteine coordination product at pH 6.0					
Scan rate: 60 mVs ⁻¹		Oxidation Peak		Reduction Peak	
[Cu] ⁺² mM	[cys] mM	E _{pa} V	I _{pa} μA	E _{pa} V	I _{pa} μA
1 ml	0.0 ml	0.318	0.441	-0.120	-0.194
1 ml	0.1 ml	0.302	0.391	-0.108	-0.164
1 ml	0.2 ml	0.286	0.358	-0.095	-0.146
1 ml	0.3 ml	0.256	0.283	-0.079	-0.120
1 ml	0.4 ml	0.223	0.222	-0.076	-0.098
1 ml	0.5 ml	0.191	0.167	-0.060	-0.081

The electrochemical behaviour of the redox couple gradually shifted from reversible to quasi-reversible characteristics, as evidenced by an increase in peak-to-peak separation (ΔE_p).^[5] This increase indicates slower electron-transfer kinetics, which may be attributed to the formation of stable copper–cysteine coordination products and surface-bound species that hinder rapid electron exchange at the electrode interface.^[4, 5, 14, 15]

Optical Characterization

UV-visible spectral analysis

The UV-visible spectrum of L-cysteine (Fig. 3 a) shows a strong absorption peak at 231 nm in the low UV region, which is attributed to the electronic transition associated with the thiol (-SH) and carboxyl (-COOH) functional groups of the amino acid.^[2] Upon coordination of the copper metal ion with the thiol group of L-cysteine in an acetate buffer at pH 6.0, significant changes in absorption spectra were recorded, including shifts in position and intensities, confirming the formation of the copper–cysteine coordination product at approximately 300 nm^[1-2] (Fig. 3b). This absorption band is possible due to metal-to-ligand charge transfer (MLCT) spectra along with d-d transitions.^[2] Following the addition of a reducing agent to the copper–cysteine coordination product, the formation of copper nanoparticles results in a broad absorption band centered at approximately 575 nm (Fig. 3c). This band is characteristic of surface plasmon resonance (SPR), which is responsible for copper nanoparticle formation.^[5-10] The UV-visible spectrum of L-cysteine-capped Cu-NPs shows a slightly blue-shifted and more stable SPR band compared to uncapped Cu-NPs, which typically show an SPR band near 580 nm (Fig. 3d). The shift indicates the successful capping of nanoparticles with L-cysteine molecules, which helps control particle size and prevents aggregation.^[1] Furthermore, the intensity, position, and width of the SPR band strongly suggest a relatively uniform particle size distribution and morphology. Spectral data show that

**Fig. 3:** UV-visible spectrum of Cu(II)–cysteine coordination product and L-cysteine-capped copper nanoparticles.

the surrounding medium, particularly the acetate buffer system and pH conditions, affects the spectral properties.

Fourier transform infrared spectroscopy

The FTIR spectrum of pure L-cysteine (Fig. 4a) shows a weak band around 2550 cm⁻¹, which corresponds to the -SH stretching vibration of the thiol (-SH) group, which is characteristic of free L-cysteine molecules. The asymmetric and symmetric stretching vibrations of the carboxylate group (COO⁻) are observed at about 1600–1620 cm⁻¹ and 1390–1410 cm⁻¹, respectively. In addition, absorption bands appearing between 1040–1080 cm⁻¹ are related to -C-N stretching vibrations.^[16] Upon coordination of copper ions with L-cysteine, significant changes are observed in the FTIR spectrum (Fig. 4b). The characteristic thiol stretching band (-SH) at approximately 2550 cm⁻¹ drastically decreases in intensity or disappears completely, revealing that the thiol group participates in copper binding by forming a Cu-S bond. Additionally, small changes in carboxylate stretching frequencies relative to the uncomplexed ligand indicate the coordination of oxygen and/or nitrogen donor atoms. The formation of the Cu(II)-S coordination product is supported by a new weak absorption band at 500–600 cm⁻¹, attributed to Cu-S and/or Cu-N vibrational modes.^[2] The FTIR spectrum supports copper coordination; however, FTIR alone does not establish a unique molecular formula, copper oxidation state, or complete coordination geometry. Copper nanoparticles show broad, relatively weak absorption bands, mainly attributed to surface-adsorbed species and partial surface oxidation. A broad absorption band around the 3200–3400 cm⁻¹ region is attributed to the O-H stretching vibration of the adsorption of water molecules or hydroxyl molecules. Because distinct bands for organic functional groups, specifically the thiol group, are absent, it can be inferred that the nanoparticle surface is mostly metallic with minimal organic coverage.^[4] The FTIR spectrum of L-Cys-CuNPs supports surface functionalization. The absence of the free SH stretching band indicates that L-cysteine's thiol groups are firmly



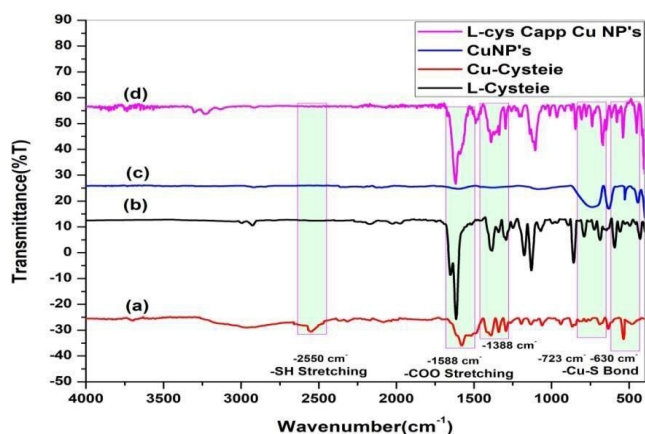


Fig. 4: FTIR spectrum of Cu(II)-cysteine coordination product and L-cysteine-capped copper nanoparticles.

bound to the copper surfaces. The COO^- asymmetric and symmetric stretching vibrations are maintained but slightly shifted, indicating that the carboxylate groups remain exposed and contribute to colloidal stability. The detection of bands around $500\text{--}700\text{ cm}^{-1}$ indicates Cu-S bond formation (Fig. 4d). The results show that L-cysteine effectively bound and stabilized the copper nanoparticles.^[10]

Structural and Crystallographic Characterization

X-ray diffraction (XRD)

X-ray diffraction (XRD) was used to investigate the crystalline structure of pure L-cysteine, the copper-cysteine coordination product, copper nanoparticles (CuNPs), and L-Cys-CuNPs. The XRD pattern of pure L-cysteine (Fig. 5a) shows sharp, intense diffraction peaks, confirming its crystalline nature. These diffraction peaks arise from an ordered molecular arrangement stabilized by intermolecular hydrogen bonding and zwitterion interactions between $-\text{NH}_3^+$ and $-\text{COO}^-$ groups.^[11] However, in contrast to previous observations, the XRD pattern of the copper-cysteine coordination product (Fig. 5b) shows weak, broadened diffraction features, indicating

reduced crystallinity and the absence of well-defined long-range structural order. The XRD results therefore support the non-crystalline nature of the isolated coordination product but do not establish the oxidation state or detailed coordination environment of copper. As a result, the diffraction pattern appears predominantly amorphous, indicating that a coordinated copper-cysteine coordination product rather than a crystalline metallic phase has formed.^[2,11] The XRD pattern of the synthesized copper nanoparticles (Fig. 5c) shows clear diffraction peaks at 2θ values of 43.3° , 50.4° , and 74.1° , corresponding to the (111), (200), and (220) crystallographic planes of face-centered cubic (fcc) metallic copper.^[17] The peak positions match the standard JCPDS data (Card No. 04-0836), confirming the successful formation of metallic Cu nanoparticles. Furthermore, the broadened diffraction peaks indicate the nanocrystalline nature of the particles. The XRD pattern of L-Cys-CuNPs (Fig. 5d) shows the same characteristic diffraction peaks corresponding to fcc copper. However, the peak intensity is slightly reduced, along with peak broadening. These changes indicate that L-cysteine acts as a capping agent, restricting crystal growth and resulting in a smaller crystallite size.^[17]

Furthermore, the absence of other impurity peaks indicates that the capping technique does not affect the copper crystalline phase. Peaks of copper oxides (Cu_2O or CuO) that are usually strong enough to be detected by XRD are not found in the diffractogram. This means that L-cysteine, by forming strong Cu-S bonds, can only partially protect copper from oxidation.^[17]

Morphological and elemental characterization

Surface morphology and particle size were characterized using scanning electron microscopy (SEM) with a TESCAN CLARA series instrument (TESCAN, Czech Republic). SEM images enabled evaluation of morphology and estimation of particle size. Particle size measurements were conducted using ImageJ software. Elemental composition was determined by energy-dispersive X-ray spectroscopy (EDS) integrated with the SEM system.

Scanning Electron Microscopy (SEM)

Copper-cysteine coordination product

An aqueous solution of copper ions is mixed with an aqueous solution of L-cysteine in acetate buffer at pH 6.0, resulting in the formation of a Cu(II)-complex. The copper ion strongly interacts with L-cysteine through the thiol ($-\text{SH}$) group, forming a Cu-S bond.^[1,3] During complex formation, particles form at different rates because no external stabilizing or capping agent is present.^[18] The synthesized Cu(II)-complex exhibited particles with various morphologies, including spherical and quasi-spherical shapes. SEM analysis shows slight particle aggregation, likely due to intermolecular interactions between the complex particles. ImageJ analysis of the

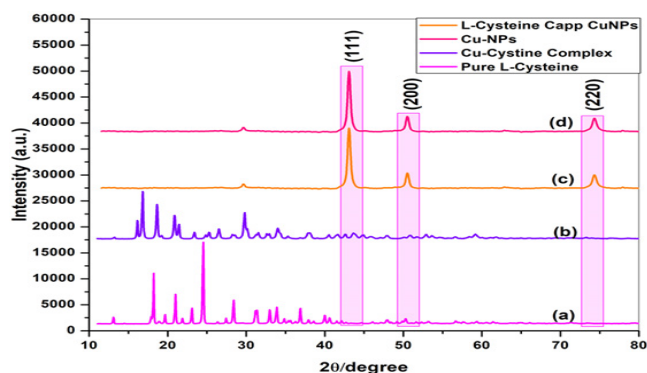


Fig. 5: XRD spectrum of Cu(II)-cysteine coordination product and L-cysteine-capped copper nanoparticles.

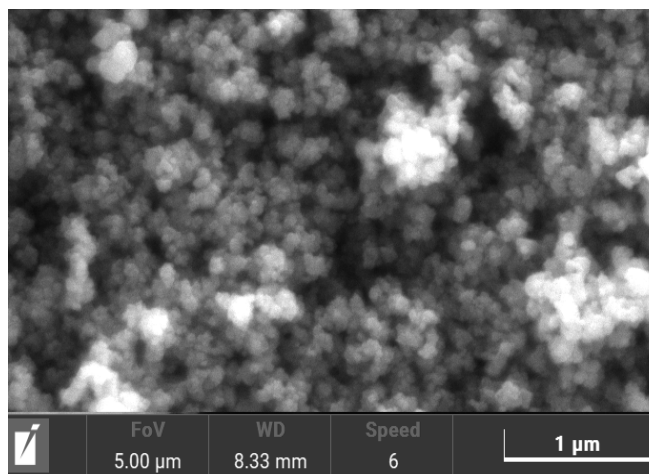


Fig. 6: SEM image of Cu(II)-cysteine coordination product showing particles with an average size of approximately 13 nm.

calibrated SEM images indicated an average particle size of approximately 13 nm for the copper–cysteine coordination product. [18]

L-cysteine-capped copper nanoparticles

Adding a small amount of reducing agent reduced the copper–cysteine coordination product to L-Cys-CuNPs. SEM analysis showed that the synthesized nanoparticles exhibited a predominantly spherical, well-dispersed morphology with slight aggregation, indicating effective stabilization by the capping ligand. [12, 18] ImageJ analysis of the calibrated SEM images indicated an average particle size of approximately 18 nm for the L-Cys-CuNPs. This increase in particle size compared to the copper–cysteine coordination product can be attributed to the growth of metallic copper nanoparticles and effective surface capping by the L-cysteine molecule. The thiol (-SH) group of cysteine effectively interacts with copper through the formation of the Cu-S bond. [1] Furthermore, L-cysteine contains functional groups such as carboxylate (-COO⁻) and amino (-NH₂) groups that remain exposed on the nanoparticle surface. These functional groups provide more steric hindrance and electrostatic repulsion between adjacent particles. [12, 18]

Energy Dispersive Spectroscopy

Copper–cysteine coordination product

The EDS spectrum of the copper–cysteine coordination product indicates high atomic percentages of carbon (65.3%), nitrogen (12.1%), and oxygen (13.6%), as well as the presence of sulfur and copper, thereby confirming the elemental composition of the synthesized complex. The high abundance of carbon, nitrogen, and oxygen is attributed to the strong organic backbone of L-cysteine, which contains amino (-NH₂), carboxyl (-COOH/-COO⁻), and thiol (-SH) functional groups. L-Cysteine

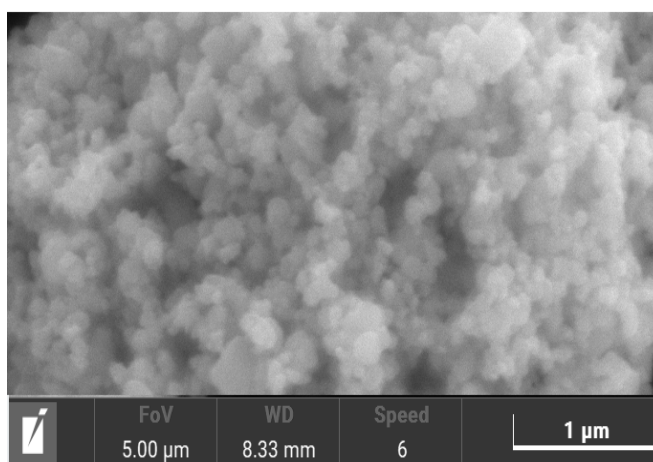


Fig. 7: SEM image of L-cysteine-capped copper nanoparticles showing particles with an average size of approximately 18 nm.

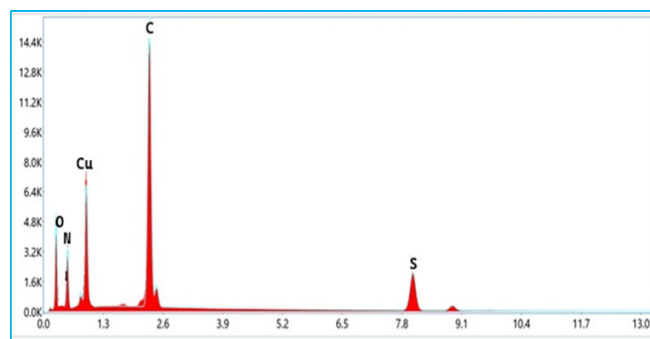


Fig. 8: EDS spectrum of the Cu(II)-cysteine coordination product showing the characteristic elemental signals of carbon (C), nitrogen (N), oxygen (O), sulfur (S), and copper (Cu)

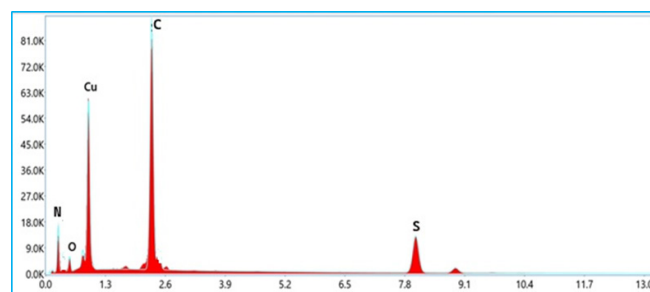


Fig. 9: EDS spectrum of L-Cys-CuNPs showing the characteristic elemental signals of carbon (C), nitrogen (N), oxygen (O), sulfur (S), and copper (Cu), confirming the presence of cysteine-associated elements and copper

is a multifunctional ligand that can coordinate metal ions through its thiol sulfur, amino nitrogen, and carboxylate oxygen donor sites. [1,19] The sulfur signal in the EDS spectrum indicates incorporation of the sulfur-containing L-cysteine ligand into the copper complex. Previous structural studies have demonstrated that Cu(II) can coordinate with L-cysteine through the thiol sulfur, with sulfur ligands participating in the copper



coordination environment.^[19] The comparatively low copper content (3.3 at.%) suggests that the synthesized material contains a ligand-rich organic coordination environment. Copper ions are incorporated within a ligand-rich coordination environment. Thus, EDS results, together with spectroscopic characterization, support the presence of copper and L-cysteine-derived coordination; however, EDS provides elemental composition and does not directly establish the oxidation state or detailed coordination geometry of copper. The Cu-S interaction is further consistent with the strong coordination ability of thiol-containing amino acids toward metal centers.^[1,19]

L-cysteine-capped copper nanoparticles

The EDS spectrum of the L-Cys-CuNPs shows the presence of high amounts of carbon (64.5 at.%), nitrogen (16.6 at.%), oxygen (5.6 at.%), and sulfur (5.6 at.%), and copper (5.2 at.%). The high carbon and nitrogen concentrations are attributed to the organic structure of L-cysteine on the nanoparticle surface. The detection of sulfur provides strong evidence for L-cysteine attachment to metallic copper nanoparticles via the thiol (–SH) group, forming Cu–S bonds.^[20] The presence of nitrogen and oxygen further suggests that L-cysteine's amino (–NH₂) and carboxylate (–COO[–]) functional groups remain attached to the nanoparticle surface, contributing to stabilization. The copper signal indicates metallic copper nanoparticles, while the higher sulfur content compared with the L-Cys-CuNPs suggests successful surface functionalization by L-cysteine.^[1,19] The copper signal supports the presence of copper in the synthesized nanoparticle. In contrast, the simultaneous detection of sulfur, nitrogen, oxygen, and carbon demonstrates the association of L-cysteine with the copper-containing nanomaterials. The relatively appreciable sulfur content (5.6 at.%) compared with the copper–cysteine coordination product indicates effective incorporation of an L-cysteine capping layer on the nanoparticle surface. Similar L-cysteine-functionalized copper nanoparticles have been reported to exhibit high stability, with L-cysteine acting as both a capping and functionalizing agent.^[20] Therefore, the EDS results support successful synthesis and L-cysteine surface functionalization of the copper nanoparticles. The EDS elemental composition revealed that the copper nanoparticles are coated with L-cysteine, which serves as the capping and stabilizing agent. These results support that the synthesis, stabilization, and capping of the copper nanoparticles were achieved through strong Cu–S interactions and surface coordination effects.^[1,19,20] however, EDS alone supports elemental composition rather than chemical state or bonding environment; therefore, complementary FTIR characterization and reported literature better support the assignment of Cu–S bonding.^[19,20]



Fig. 10: ZOI of copper–cysteine coordination product and cysteine-capped copper nanoparticles against *E. coli*.



Fig. 11: ZOI of Cu(II)–cysteine coordination product and cysteine-capped copper nanoparticles against *S. aureus*.

Antibacterial activity Studies

Antibacterial activity against Gram-positive bacteria: *Staphylococcus aureus*

The antibacterial activity of the Cu(II)-cysteine-coordinated product and L-Cys-CuNPs was tested against the Gram-positive bacterium *Staphylococcus aureus* using the agar well diffusion method. The Cu(II)-cysteine coordination product had a zone of inhibition (ZOI) of 11.63 ± 0.63 mm, while L-Cys-CuNPs showed a ZOI of 15.07 ± 0.07 mm against *S. aureus* ($n = 3$ independent measurements; Fig. 11). Results are shown as mean $\pm$ standard deviation. In these experiments, the Cu(II)-

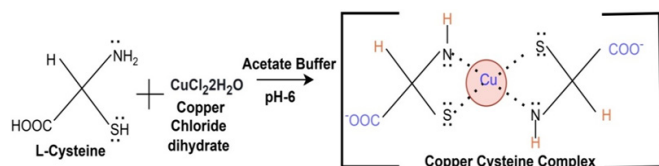


Fig. 12: Proposed coordination structure of the Cu(II)-cysteine coordination product formed in sodium acetate buffer at pH 6.0

Table 2: Zone of inhibition (ZOI) of Cu(II)-cysteine coordination product and L-cysteine-capped copper nanoparticles against *E. coli* and *S. aureus*

Sample	Replicate 1	Replicate 2	Replicate 3	± SD
Cu(II)-cysteine — <i>E. coli</i>	12	11.9	12.3	12.07 ± 0.21
L-Cys-CuNPs — <i>E. coli</i>	16.15	15.9	16.1	16.05 ± 0.13
Cu(II)-cysteine — <i>S. aureus</i>	11.00	11.6	12.3	11.63 ± 0.63
L-Cys-CuNPs — <i>S. aureus</i>	15.15	15.05	15.02	15.07 ± 0.07

cysteine coordination product was tested against *S. aureus*. Differences in the materials' physicochemical and surface properties may help explain the antibacterial effects seen here. This study did not directly examine copper release, membrane damage, protein denaturation, ROS production, or the specific role of Cu-S interactions. These mechanisms are mentioned only as possible explanations from the literature and are not experimentally confirmed in this work.

Antibacterial activity against Gram-negative bacteria: Escherichia coli

The antibacterial activity against the Gram-negative bacterium *Escherichia coli* was tested using the agar well diffusion method. The Cu(II)-cysteine coordination product had a zone of inhibition (ZOI) of 12.07 ± 0.21 mm, while L-Cys-CuNPs showed a ZOI of 16.05 ± 0.13 mm (SD) (Fig. 10). Results are shown as mean ± standard deviation (SD). In these experiments, the Cu(II)-cysteine coordination product produced a slightly smaller inhibition zone than L-Cys-CuNPs against *E. coli*. This difference may be due to variations in the physicochemical properties and surface features of the two copper-containing materials. Earlier studies have found that copper-based materials can have antibacterial effects by interacting with bacterial surfaces and through oxidative stress processes.^[8] However, this study did not directly measure copper release, membrane damage, or ROS generation, so these mechanisms are only possible explanations based on previous reports and were not confirmed experimentally.

The antibacterial activity of the Cu(II)-cysteine coordination product and L-Cys-CuNPs was assessed

against the Gram-positive bacterium *Staphylococcus aureus* and the Gram-negative bacterium *Escherichia coli* using the agar well diffusion method. Test samples were prepared at a concentration of 1 mg/mL, and equal volumes were dispensed into wells of approximately 6 mm diameter in Mueller-Hinton agar plates. Sterile water was used as the negative control, and streptomycin (1 mg/mL) as the positive control under identical conditions. Plates were incubated at 37°C for 24 hours, and zones of inhibition (ZOI) were measured in millimetres (mm). Data were collected from three independent experiments (n = 3) and are reported as mean ± standard deviation (SD).

Proposed structures

Copper-cysteine coordination products were synthesized by combining copper with the L-cysteine amino acid in sodium acetate buffer under slightly acidic conditions (pH 6.0). The formation of the copper-cysteine coordination product is governed by the strong coordination affinity of copper ions to the thiol (-SH), amine (-NH₂), and carboxylate (-COO⁻) functional groups of L-cysteine. At this pH, L-cysteine exists in a zwitterionic form, which facilitates effective chelation through the sulfur and nitrogen donor groups, leading to a stable copper-cysteine coordination product (Fig. 12).^[21-22] The acetate buffer helps maintain the reaction pH and provides a controlled medium for copper-cysteine coordination.^[22]

Spectroscopic and electrochemical studies show that L-cysteine coordination significantly alters the redox behaviour of the copper ion; these changes are reflected in shifts in peak potentials and variations in peak current, resulting from reduced availability of free copper ions in solution due to complex formation.^[23] The thiol group of L-cysteine plays a dominant role in coordination by forming a Cu-S bond, which enhances complex stability under suitable conditions.^[23-24]

CONCLUSION

As L-cysteine was added to the copper solution, the peak potential and peak current decreased, indicating the formation of the copper-cysteine coordination product. UV-visible and FTIR analyses supported copper-L-cysteine interaction through the thiol, amino, and carboxylate functional groups. L-Cys-CuNPs are formed through the thiol (-SH), amino (-NH₂), and carboxylate (-COO⁻) functional groups. FTIR analysis supports that L-cysteine interacts strongly with both copper ions and copper nanoparticles in acetate buffer at pH 6.0.

The disappearance or weakening of the thiol stretching vibration and the appearance of bands attributed to Cu-S and Cu-N interactions support L-cysteine's involvement in copper coordination. XRD analysis confirmed the crystalline, single-phase nature of the synthesized copper nanoparticles. The diffraction peaks corresponding to the face-centered cubic structure of metallic copper



confirmed successful formation of the crystalline phase. after capping, this indicated that L-cysteine stabilized the nanoparticles without altering their crystal structure in acetate buffer at pH 6.0. The effective synthesis and stability of the copper–cysteine coordination product and L-Cys-CuNPs were confirmed by scanning electron microscopy (SEM), where SEM was used to examine the surface morphology and particle size of the synthesized materials. SEM images highlighted the role of L-cysteine in controlling particle morphology and increasing dispersion, showing nearly spherical, well-dispersed nanoparticles with less agglomeration. EDS analysis confirmed the elemental composition of the synthesized copper-cysteine coordination product and L-Cys-CuNPs, showing the characteristic C, N, O, S, and Cu elements. These results support the presence of cysteine-associated components in the copper-containing material. Overall, the results show that adding L-cysteine capping influences the surface characteristics, particle morphology, and dispersion of copper nanoparticles under the experimental conditions used. The antibacterial evaluation showed that both copper-containing materials exhibited inhibitory activity against the tested *E. coli* and *S. aureus* strains. Under the experimental conditions used, the Cu(II)-cysteine coordination product showed slightly larger inhibition zones than L-Cys-CuNPs for both tested bacteria. However, the present study did not directly investigate the specific molecular mechanism responsible for the observed antibacterial activity, which requires further experimental investigation. The proposed structure of the copper-cysteine coordination product represents the possible interaction of copper with the thiol, amino, and carboxylate donor groups of L-cysteine under the experimental conditions at pH 6.0. The combined electrochemical, spectroscopic, and elemental characterization results support the proposed structure; however, the exact molecular formula and coordination geometry could not be conclusively established.

REFERENCES

- Yoshinari N, Kuwamura N, Kojima T, Konno T. Development of coordination chemistry with thiol-containing amino acids. *Coord Chem Rev.* 2023;474:214857. Available from: <https://doi.org/10.1016/j.ccr.2022.214857>
- Rubino JT, Franz KJ. Coordination chemistry of copper proteins: How nature handles a toxic cargo for essential function. *J Inorg Biochem.* 2012;107:129–143. Available from: <https://doi.org/10.1016/j.jinorgbio.2011.11.024>
- Falcone E, Hureau C. Redox processes in Cu-binding proteins: The “in-between” states in intrinsically disordered peptides. *Chem Soc Rev.* 2023;52(19):6595–6600. Available from: [10.1039/D3CS00443K](https://doi.org/10.1039/D3CS00443K)
- Bard AJ, Faulkner LR. *Electrochemical Methods: Fundamentals and Applications*. 2nd ed. New York: Wiley; 2001. Available from: [10.1002/0471043729](https://doi.org/10.1002/0471043729)
- Kissinger PT, Heineman WR. Cyclic voltammetry. *J Chem Educ.* 1983;60:702–706. Available from: [10.1021/ed060p702](https://doi.org/10.1021/ed060p702)
- Wang J. *Analytical Electrochemistry*. 3rd ed. Hoboken, NJ: Wiley-VCH; 2006. Available from: <https://doi.org/10.1002/0471790303>
- Coates J. Interpretation of infrared spectra: A practical approach. In: Meyers RA, editor. *Encyclopedia of Analytical Chemistry*. Chichester: Wiley; 2006. Available from: [10.1002/9780470027318.a5606](https://doi.org/10.1002/9780470027318.a5606)
- Lemire JA, Harrison JJ, Turner RJ. Antimicrobial activity of metals: Mechanisms, molecular targets and applications. *Nat Rev Microbiol.* 2013;11:371–384. Available from: [10.1038/nrmicro3028](https://doi.org/10.1038/nrmicro3028)
- Lide DR, editor. *CRC Handbook of Chemistry and Physics*. 100th ed. Boca Raton, FL: CRC Press; 2020. Available from: [10.1201/9780429340491](https://doi.org/10.1201/9780429340491)
- Soomro RA, Nafady A, Sirajuddin, Memon N, Sherazi TH, Kalwar NH. L-Cysteine-protected copper nanoparticles as colorimetric sensor for mercuric ions. *Talanta.* 2014;130:415–422. Available from: <https://doi.org/10.1016/j.talanta.2014.07.023>
- Moggach SA, Allan DR, Clark SJ, Gutmann MJ, Parsons S, Pulham CR, Sawyer L. High-pressure polymorphism in L-cysteine: The crystal structures of L-cysteine-III and L-cysteine-IV. *Acta Crystallogr B Struct Sci.* 2006;62(2):296–309. Available from: <https://doi.org/10.1107/S0108768105038802>
- Din MI, Arshad F, Hussain Z, Mukhtar M. Green adeptness in the synthesis and stabilization of copper nanoparticles: Catalytic, antibacterial, cytotoxicity, and antioxidant activities. *Nanoscale Res Lett.* 2017;12:638. Available from: [10.1186/s11671-017-2399-8](https://doi.org/10.1186/s11671-017-2399-8)
- Sigel H, Sigel A, editors. *Metal Ions in Biological Systems: Interactions of Metal Ions with Amino Acids*. New York: Marcel Dekker; 1996.
- Rulišek L, Vondrášek J. Coordination geometries of selected transition metal ions (Co²⁺, Ni²⁺, Cu²⁺, Zn²⁺, Cd²⁺, and Hg²⁺) in metalloproteins. *J Inorg Biochem.* 1998;71:115–127. Available from: [10.1016/S0162-0134\(98\)10042-9](https://doi.org/10.1016/S0162-0134(98)10042-9)
- Li YR, Trush MA. Defining reactive oxygen species in biology and medicine. *React Oxyg Species (Apex)*. 2016;1:9–21. Available from: <https://doi.org/10.20455/ros.2016.803>
- Socrates G. *Infrared and Raman Characteristic Group Frequencies: Tables and Charts*. 3rd ed. Chichester: Wiley; 2001.
- Cullity BD, Stock SR. *Elements of X-Ray Diffraction*. 3rd ed. Upper Saddle River, NJ: Prentice Hall; 2001.
- Nasrollahzadeh M, Sajadi SM, Sajjadi M, Issaabadi Z. An introduction to green synthesis of metal nanoparticles. *J Colloid Interface Sci.* 2019;538:126–142. Available from: [10.1016/j.jcis.2018.11.065](https://doi.org/10.1016/j.jcis.2018.11.065)
- Dokken KM, Parsons JG, McClure JC, Gardea-Torresdey JL. Synthesis and structural analysis of copper(II) cysteine complexes. *Inorganica Chimica Acta.* 2009;362(2):395–401. Available from: <https://doi.org/10.1016/j.ica.2008.04.037>
- Kumar N, Upadhyay LSB. Facile and green synthesis of highly stable L-cysteine functionalized copper nanoparticles. *Applied Surface Science.* 2016;385:225–233. Available from: <https://doi.org/10.1016/j.apsusc.2016.05.125>
- Kaim W, Schwederski B. *Bioinorganic Chemistry: Inorganic Elements in the Chemistry of Life: An Introduction and Guide*. Wiley: Chichester; 2013. Available from: <https://doi.org/10.1002/9781118651197>
- Bagchi P, Das S. Coordination Chemistry of Copper with Thiol-Containing Amino Acids: Structure and Reactivity. *Coord Chem Rev.* 2020;422:213479. Available from: <https://doi.org/10.1016/j.ccr.2020.213479>
- Santoro A, et al. Copper(II) Binding to Cysteine and Related Thiol Ligands: A Spectroscopic and Electrochemical Study. *Inorg Chem.* 2019;58:14503–14512. Available from: <https://doi.org/10.1021/acs.inorgchem.9b0212>
- Hureau C, et al. Electrochemical study of Cu(II)–cysteine complexes in aqueous solution. *Electrochimica Acta.* 1990;35(1):47–53. Available from: [https://doi.org/10.1016/0013-4686\(90\)85034-K](https://doi.org/10.1016/0013-4686(90)85034-K)

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